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In Vitro Study of Fermented Complete Feed by Using Sago Residues as Main Source Diet

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Abstract. Recently, fermentation of low quality feed such as agro industry by products has been widely applied to produce enriched animal feed and improve animal productivity. The purpose of this study was to evaluate in vitro digestibility of fermented complete feed using agro-residues from sago starch processing industries as main source diet. Feed was formulated on the basis of 40% sago residue and mixed with other ingredients (rice brand, coconut meal, bread by product, soybean meal and soybean hulls) to fulfill the requirement of sheep with 16.10% of CP, 2.80 Mcal ME/kg and TDN 60.88% based on calculation. Mineral and Urea were added in the complete feed to reach mineral and CP requirements of sheep. Complete feed formulation was fermented by using 3 commercial fermentation products (Saus Burger Pakan® (SBP), Probion® and EM4®) for 21 days. Total and types of microbes from commercial fermentation products utilized in this study were not determined. Parameters measured in this study were pH, NH_3, IVDMD (in vitro dry matter digestibility), IVOMD (in vitro organic matter digestibility) and VFA total. The model used for the statistical analysis was completely randomized design (CRD) with 4 treatments (control and 3 different commercial fermentation products) and 4 replications. Result indicated that administration of different commercial fermentation products into fermented complete feed based on sago residues significantly influenced ($P<0.05$) on pH, NH3, VFA total, IVDMD and IVOMD. Generally, commercial fermentation products mainly SBP produced better feed quality by improving the values of pH, NH3, VFA total, IVDMD and IVOMD. However, types and total microorganisms needed to be determined prior to experiment.

Key Words: fermentation, sago waste, in vitro, complete feed

Abstrak. Akhir-akhir ini penggunaan bahan pakan berkualitas rendah yang berasal dari limbah industri pertanian telah diterapkan secara luas yang bertujuan untuk meningkatkan kualitas pakan dan produktivitas ternak. Tujuan dari penelitian ini adalah untuk mengevaluasi *complete feed* fermentasi berbahan dasar ampas sagu secara *in vitro*. Pakan diformulasikan dengan menggunakan 40% ampas sagu dan dicampur dengan bahan pakan lainnya (dedak, bungkil kelapa, sisa roti, bungkil kedelai dan kulit ari kedela) untuk memenuhi kebutuhan nutrisi domba (16,10% of PK, 2,80 Mkal ME/kg and TDN 60,88%). Mineral adan urea ditambah pada *complete feed* untuk memenuhi kebutuhan mineral dan protein dari ternak domba. *Complete feed* yang telah diformulasi difermentasi dengan menggunakan 3 produk fermentasi komersil (Saus Burger Pakan® (SBP), Probion® and EM4®) selama 21 hari. Total dan jenis mikro organisme dalam produk fermentasi komersil tidak dihitung. Penelitian ini menggunakan rancangan acak lengkap yang terdiri dari 4 perlakuan dan 3 ulangan. Parameter yang dihitung dalam penelitian ini adalah pH, NH_3, total VFA dan kecernaan bahan kering dan kecernaan bahan organik. Hasil penelitian menunjukan bahwa fermentasi *complete feed* dengan menggunakan produk fermentasi komersial berpengaruh nyata ($P<0.05$) terhadap pH, NH_3, total VFA dan kecernaan bahan kering dan kecernaan bahan organik. Secara umum bahan produk fermentasi komersil terutama SBP dapat meningkatkan kualitas pakan dengan memperbaiki nilai pH, NH_3, total VFA dan kecernaan bahan kering dan kecernaan bahan organik. Namun penentuan jumlah dan jenis mikro organisme perlu dilakukan sebelum penelitian dilakukan.

Key Words: fermentasi, ampas sagu, *in vitro*, *complete feed*

Introduction

It is well known that feed plays an important factor in animal production (Samadi and Liebert, 2008; Samadi, 2012) and spends about 70% of production cost in livestock enterprise. The use of low external input agricultural system coming from agro industry by products is able to reduce feed cost. However, feed from agro and industry by products contains lower

nutritive values and digestibility. Several methods can be applied to improve nutrient content and digestibility of feed (Bisaria et al., 1997; Tang et al., 2008; Wanapat et al., 2009; Rahman et al., 2011; Shrivastava et al., 2011; Wajizah et al., 2015).

One of the agro industry by products that can be utilized as animal feed is agro-residues from sago starch processing industries which is abundant and readily available in Indonesia. Tampoebolon (2009) stated that sago waste contain 28.30% crude fiber and only 1.36% crude protein. According to Linggang et al. (2012) sago residues after the starch extraction process contain starch (58%), cellulose (23%), hemicellulose (9.2%) and lignin (3.9%). The high content of crude fiber and lignin results in slow and limited ruminal degradation of the carbohydrates in the rumen. In addition, the low content of nitrogen is the main deficiencies from agro residues causing the low value of residues as feed for ruminants (Van Soest, 2006).

In past years, many studies have been carried out to improve low quality residues as animal feed with various methods such as physics (Cristiyanto and Subrata, 2005; Viola et al., 2008; Samadi and Yu 2011; Samadi et al., 2013), chemistry (Bata, 2008) and biology (Pandey et al., 2000; Tang et al., 2008; Wajizah et al., 2015). Feed technology fermentation using microbial appears to be a practical and promising alternative to improve nutritional value of agro residues by processing these materials into animal feed and thus resulting in a value-added product. Research conducted by Zadrazil and Puniya (1995) by fermentation of sugarcane bagasse with white-rot fungi was able to improve the bagasse digestibility with all fractions and produce enriched animal feed. The purpose of this study was to evaluate in vitro digestibly of fermented complete feed by using agro-residues from sago starch processing industries as main source diet.

Materials and Method

Sago residues and diet formulation

Sago residue utilized in this study was collected from sago starch processing industry located in Krueng Mane, North Aceh and dried up to 10% of DM. Feed was formulated on the basis of 40% sago residue and mixed with other ingredients (rice brand, coconut meal, bread by product, soybean meal and soybean hulls) to fulfill the requirement of sheep (16.10% of CP, 2.80 Mkal ME/kg and TDN 60.88%) (SNI and NRC, 2007). Mineral and Urea were added in the complete feed to reach mineral and CP requirements of sheep. Complete feed formulation was fermented using 3 commercial fermentation products (Saus Burger Pakan® (SBP), Probion® and EM4®). These commercial fermentation products like SBP consists of various microorganisms such as cellulolytic, lactic acid, amylolytic microbes (Wulandari et al., 2014[b]). Each treatment was added 0.3% of commercial fermentation product (except for control treatment) with 60% DM by adding water. Prior to fermentation, microorganisms from commercial products were activated in the 2 % of molasses solution for 2 hours (Wulandari et al., 2014[b]). Samples were kept for anaerobe fermentation at room temperature for 21 days. The feed formulation for in vitro study is shown in Table 1.

Chemical analysis

Chemical analysis was conducted at Animal Nutrition Laboratory of Bogor Institute Agriculture and Animal Nutrition Laboratory of Syiah Kuala University, Banda Aceh. The method to measure in vitro analysis was based on Tilley dan Terry (1963) in which samples were incubated for 24 hours and continued for post rumen digestibility by adding of pepsin for the next 24 hours. Concentration of pH, N-NH_3

Table 1. Composition of experimental diet based on sago residues as main ingredients (% as fed)

Ingredients	Treatment*			
	F_0 (Control)	F_1(SBP)	F_2(Probion)	F_3(EM-4)
Sago residue	40	40	40	40
Rice brand	18.5	18.2	18.2	18.2
Coconut meal	18	18	18	18
Bread by product	2.5	2.5	2.5	2.5
Soybean meal	8	8	8	8
Soybean hulls	8	8	8	8
Urea	2	2	2	2
Molasses	1.5	1.5	1.5	1.5
NaCl	0.5	0.5	0.5	0.5
Mineral*	1	1	1	1
Commercial Fermentation Product**	0	0.3	0.3	0.3
TOTAL	100	100	100	100

*F_0= Control (without fermentation); F_1= Fermentation with SBP; F_2=Fermentation with Probion and F_3= Fermentation with EM-4

and VFA total for in vitro analysis was determined according to Soejono (1996). Measurement of rumen pH was conducted at the end of every incubation period. Total VFA concentration was determined based on steam distillation and N-NH_3 concentration was measured by micro diffuse convey technique (General Laboratory Procedure, 1966).

Statistical analysis

Statistical analyses (P≤0.05) used Statistical Software Package SPSS. The model used for the analysis was completely randomized design (CRD) with 4 treatments (control and 3 different commercial fermentation products) and 4 replications. The following is formula applied for statistical analysis: $Yij = \mu + Ti + eij$, where, Yij was an observation of the dependent variable ij; μ was the population mean for the variable; Ti was the effect of the fermentation, as a fixed effect, and eij was the random error associated with the observation ij. Differences between variables were compared by a one-way analysis of variance (ANOVA). Verification of variance homogeneity and identification of statistical significance was applied by Duncan multiple range test. Observations with (P≤0.05) were considered statistically significant and trends were declared at P≤0.10.

Results and Discussion

pH value

The pH value was considerably important to be measured due to rumen environmental condition. pH value can be used as indicator for feed degradation in which the range of pH value from 6.9-7.0 was optimum for cellulolytic microbes to degrade feed in rumen (Jean-Blain, 1991). If pH was lower than 6.2, cellulolytic microbes was disturbed and not able to grow optimally (Piwonka dan Firkins, 1996). The dynamic value of pH was an indication of feed level hydrolysis in which reducing of pH value had correlation with increasing of N microbes and increasing of total and partial VFA (Alltech, 2012). The result of pH value from this study is shown in the Figure 1.

The results of study showed that application of different commercial fermentation products for fermented complete feed had significant influence (P<0.05) on pH value of treatments. In this study, pH ranged from 6.79 to 7.0 and it was ideal condition for activity of cellulolytic microbes in rumen (Erdman, 1988). The value of pH from SBP was the lowest and

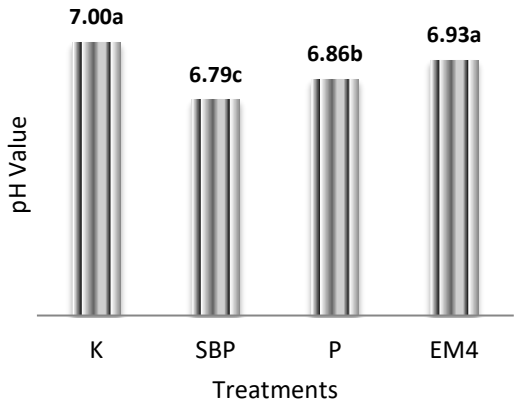

Figure 1. In vitro pH value of fermented complete feed based on sago residues as main ingredient feed with different commercial fermentation products. (K (Control); SBP (Saus Burger Pakan); P (Probion); EM4 (Efektif Mikroorganisme-4)

followed by probion, EM4 and control with 6.79, 6.86, 6.93 and 7.0 respectively. It was indicated that microbes from SBP and probion were effective to degrade structural carbohydrate from sago residues to be non-structural carbohydrate and easily digested to produce VFA and other organic acids. Czerkawski (1998) stated that enzymes hydrolyzed non-structural carbohydrates first before structural carbohydrates.

Syahrir (2009) reported that pH rumen liquor reduced in the glucose, maltose and sucrose from 7.1 to 6.5 after a 4-hour fermentation. However, when tested in the same treatment using lactose, amylum and cellulose, pH decreased slowly from 7.1 to 6.9 after a 4-hour fermentation. Different results have been reported by Sangadji (2009) using sago waste fermented with fungi *(Pleurotus ostreatus)* to substitute natural grass in complete feed. In this study, pH was not significantly effective (P>0.05) between treatments. It was probably due to high nutrient contents of diet between treatments in which the diet contained 40% of concentrate.

NH_3 concentration

NH_3 (ammonia) concentration in the rumen can be used as one of parameters to know the treatment effects on protein degradation. In the rumen, protein was degraded to peptide and amino acids and then degraded by microbes to ammonia. When protein was difficult to be degraded or in case of protein deficiency, ammonia concentration in the rumen was low and resulted in slow microbe growth (Wiryawan et al., 1999). NH_3 concentration from this study is shown in the Figure 2.

The results from this study indicated that administration of different commercial fermentation products into fermented complete feed based on sago residues significantly influenced (P<0.05) ammonia concentration after a 6-hour incubation. The highest concentration of NH_3 was SPB, followed by probion, EM-4 and control with 32.47 mM, 30.59 mM, 29.4 mM and 24.42 mM respectively. Mc. Donald et al., (2002) stated that optimum ammonia concentration for microbe protein synthesis in the rumen liquor was 5–17.65 mM. NH_3 concentration was high in all treatments, because ammonia at in vitro test was only utilized by microbes and not absorbed by rumen wall and excreted by urine. Therefore, ammonia was accumulated during in vitro test. In accordance with Hilakore (2008), fermentation putak with *Aspergillus niger* and *Trichoderma reseei* had 19, 09 mM ammonia concentration.

Previous experiment reported by Thalib et al. (2001) probion produced lower ammonium compared to probiotics contain cellulolytic bacteria like SBP. Increase of ammonia concentration by administration SBP, probion and EM-4 was as indication of the high soluble protein and enzyme degraded activities, proteololytic enzymes, from probiotic in degradation of protein (Dutta et al., 2001). In addition, increase of ammonia concentration was probably due to high ferment ability of fermented complete feed based on sago residues as a point of protein evaluation in ruminant animals.

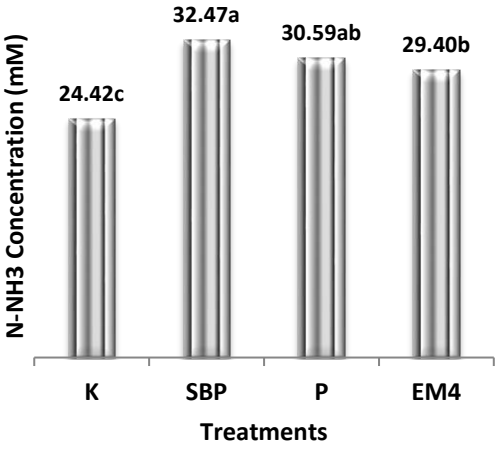

Figure 2. NH_3 concentration of fermented complete feed based on sago residues as main ingredient feed with different commercial fermentation products. (K (Control); SBP (Saus Burger Pakan); P (Probion); EM4 (Efektif Mikroorganisme-4).

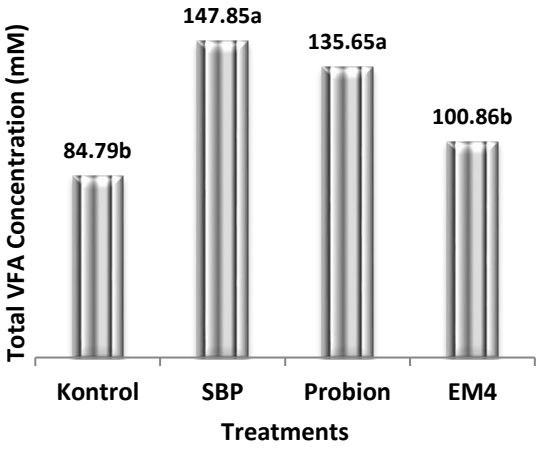

Figure 3. VFA concentration of fermented complete feed based on sago residues as main ingredient feed with different commercial fermentation products. (K (Control); SBP (Saus Burger Pakan); P (Probion); EM4 (Efektif Mikroorganisme-4).

However, lower ammoniac concentration in rumen reflected a good fermentation process and ammonia was able to be optimally utilized for animal productivity. Rumen microbes can use NPN such as urea and ammonia to formulate protein (Krause, 2001). Protein is considered a very important nutrition or growth and rumen microorganism to digest cellulose and as protein source for animals (Mc Donald et al., 2002). Low ammoniac concentration was indicated as either low degradation of protein or low protein concentration in diet. The last two reasons were assumed as low ammoniac content in rumen liquor, followed by low VFA concentration and digestibility (Syahrir, 2009).

VFA concentration

Carbohydrate digestibility in rumen produces VFA as main product and be used as main carbon bone for rumen bacteria and also energy for ruminant animals (Russell dan Wallace, 1997). VFA concentration is one or the indicators to measure fermentable feed with closely related to rumen microbe population. The amount of VFA production was affected by digestibility and fermented feed quality (Baldwin, 1995). VFA concentration from this study is shown in the Figure 3.

The results from this study shown that administration of different commercial fermentation products into fermented complete feed based on sago residues significantly influenced (P<0,05) on total VFA concentration after a-6 hour incubation. The highest total VFA was SBP and probion,147.85 mM, 135.65 mM, respectively. But VFA total for control and EM-4 were not significantly different (P>0, 05). Concentration of VFA total in this study was sufficient for rumen microbe growth since more than normal condition as stated by Waldron et al. (2002) with the number of 60-120 mM. Application of probiotic was able to influence rumen ecosystem such as pH, fermentation products such as VFA and NH3. Generally, probiotic improves VFA total production (Oeztuerk et al., 2005) as reported by Dawson dan Newman (1988) in vitro experiment.

Widyastuti (2008) stated that feeding animal with fermented feed offers chance for microorganisms to grow including LAB. The results from several in vivo studies with cattle fed on elephant grass silage with or without bacteria as inoculum showed differences on feed intake, bacteria population in rumen and

VFA concentration. However, feed intake of cattle treated with bacteria inoculum consumed lower silage but total rumen bacteria, cellulolytic bacteria and propionate: acetate ratio were improved. It means that the use of energy was more efficient.

In this study, improvement of VFA total was linier with urea concentration for all treatments. VFA production was highest at SBP supported by ammonia availability and it is indicated that improvement of complete feed quality based on sago residues was able to optimize rumen fermentation. With ammonia, VFA play very important factors for optimum microbe growth and main source to perform microbe protein to be used for animals growth. Most of rumen microbes utilize ammonium for proliferation microba mainly for protein synthesis. Meanwhile, carbohydrate was hydrolyzed to be VFA as energy source (Suryahadi dan Amrullah, 1989).

IVDMD (in vitro dry matter digestibility) and IVOMD (in vitro organic matter digestibility)

Feed digestibility is defined as feed not excreted in feces and assumed to be absorbed by animals. Digestibility can be measured by in vitro technique presented in dry matter (Mc. Donald et al., 2002; Perry et al., 2003). Nutrient digestibility is one of indications to measure feed quality since it reflected the availability of nutrient for animals. Level of feed digestibility in rumen was affected by feed chemical composition mainly the content of protein and crude fiber, rumen fermentation condition including pH, ammonia concentration and VFA supported digestibility during fermentation. Increase of feed digestibility resulted in higher VFA concentration as indication of improving animal productivity in which VFA is sources of energy for animals (Syahrir, 2009). The amount of VFA production was affected by digestibility and fermented feed quality (Baldwin, 1995). Figure 4 is shown IVDMD and IVDOM of fermented complete feed based on sago residues as main ingredient feed with different commercial fermentation products.

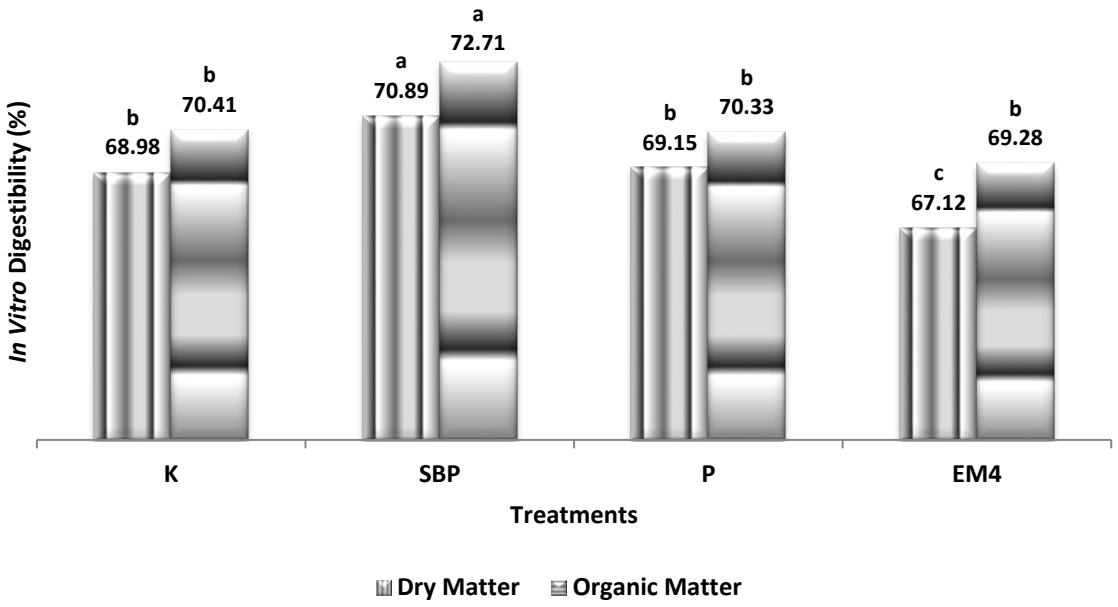

Figure 4. IVDMD and IVDOD of fermented complete feed based on sago residues as main ingredient feed with different commercial fermentation products. (K (Control); SBP (Saus Burger Pakan); P (Probion); EM4 (Efektif Mikroorganisme-4).

The results of this study showed that administration of different commercial fermentation products into fermented complete feed based on sago residues significantly influenced (P<0.05) on the percentage of IVDMD and IVDOD. SBP is the highest of all treatments with 70.89% and 72.71% IVDMD and IVDOD, respectively. Meanwhile, three other treatments were not significantly different (P>0.05) on the percentage of IVDMD and IVDOD as shown in Figure 4.

The highest percentage of IVDMD and IVDOD from SBP because the inoculum of SBP consists of mixture of BAL, bacteria cellulolytic and amylotytic which has a role in reducing of pH and decreased crude fiber from cellulolytic bacteria (Wulandari et al., 2014[a]). In the ruminant diet, probiotic is able to increase total nitrogen enter to intestine and improved the ruminant ability to degrade cellulose and hemicellulose (Yoon and Stern, 1995). Improving in vitro digestibility was due to cellulolytic enzyme produced during fermentation process. Cellulolytic enzyme is complex enzyme gradually cutting at the β-1, 4 glycosidic bond. This enzyme was able to cut cellulose chain to be cellebiose and finally produced glucose (Lin et al., 2012). Wulandari et al. (2014[b]) reported that even though there did not improve IVDMD and IVDOD from treatments, fermented complete feed based on cacao pod increased (P<0.05) crude fiber digestibility. Crude fiber can influence total digestibility since crude fiber is one of the organic components which is difficult to be digested.

In addition, Wina (2005) reported that replacement of concentrate from fermented products resulted in increasing of total feed intake, dry matter digestibility, nitrogen retention and intake N-microbes. Mechanism of digestible improvement from fermented feed was due to fermentation products that can be used by rumen microbes for growing and improve rumen to digest feed. In accordance with the research conducted by Hilakore (2008) feeding fermented *putak* 20% into concentrated improved dry matter digestibility and continued to increase up to 40% fermented *putak* in concentrate. It was concluded that fermented *putak* improved diet for animal growth. DMD was closely correlated with OMD because organic matter is the highest composition in dry matter. Decreasing of DMD results in reducing of DOM (Parakkasi, 1999).

Conclusions

In conclusion, administration of different commercial fermentation products into fermented complete feed based on sago residues significantly influenced (P<0.05) on pH, NH3, VFA total, IVDMD and IVOMD. Generally, commercial fermentation products mainly SBP produced better feed quality by improving the values of pH, NH3, VFA total, IVDMD and IVOMD. However, types and total microorganisms were needed to be determined before experiment.

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